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Evaluation of propargyl bromide for control of barnyardgrass and *Fusarium oxysporum* in three soils

Qingli Ma,[1] Jianying Gan,[1]* J Ole Becker,[2] Sharon K Papiernik[1] and Scott R Yates[1]

[1] *George E Brown Jr Salinity Laboratory, USDA-ARS, 450 West Big Springs Road, Riverside, CA 92507, USA*

[2] *Department of Nematology, University of California, Riverside, CA 92507, USA*

Abstract: With the scheduled phasing out of methyl bromide, there is an urgent need for alternatives. We evaluated the efficacy of propargyl bromide as a potential replacement for methyl bromide for the control of barnyardgrass (*Echinochloa crus-galli*) and *Fusarium oxysporum* in an Arlington sandy loam, a Carsitas loamy sand and a Florida muck soil. Soil was mixed with barnyardgrass seeds or *F oxysporum* colonized on millet seeds, and treated with propargyl bromide at a range of concentrations. The mortality of the fungi and weed seeds was determined after 24 h of exposure at 30 °C. The concentrations required to inhibit 50% barnyard seed germination (LC_{50}) were 2.8, 2.4 and 48.5 $\mu g\ g^{-1}$ in the sandy loam, loamy sand and muck soil, respectively. In contrast, the LC_{50} values for *F oxysporum* were 11.2, 10.8 and 182.1 $\mu g\ g^{-1}$ in the sandy loam, loamy sand and muck soil, respectively. The low efficacy of propargyl bromide in the muck soil was a result of the rapid degradation and high adsorption of the compound in the soil. The degradation half-life ($t_{1/2}$) was only 7 h in the muck soil at an initial concentration of 6.8 $\mu g\ g^{-1}$, compared to 60 and 67 h in the sandy loam and loamy sand, respectively. The adsorption coefficients (K_d) were 0.96, 0.87 and 5.6 $cm^3\ g^{-1}$ in the sandy loam, loamy sand and muck soil, respectively. These results suggest that registration agencies should consider site-specific properties in recommending application rates for propargyl bromide.

Keywords: propargyl bromide; efficacy; barnyardgrass; *Fusarium oxysporum*; adsorption; degradation; soil

1 INTRODUCTION

In California and Florida, field production of fresh market vegetable and fruit crops relies heavily on the use of fumigants to provide control of soil-borne pests, pathogens and weeds. Methyl bromide is a broad-spectrum fumigant and has been used extensively over the last half-century.[1] However, concerns about the possible contributions of methyl bromide to stratospheric ozone depletion have prompted a regulatory action for a complete ban of its production and importation into the USA by 2005. The National Agricultural Pesticide Impact Assessment Program (NAPIAP) has concluded that substantial adverse economic impacts would be imposed on the agricultural community without alternative fumigants.[2] As a result, there has been an increasing emphasis on finding effective, broad-spectrum, environmentally friendly substitutes for methyl bromide.

Methyl iodide has been found to be as effective as methyl bromide for controlling a variety of soil-borne pests, pathogens and weeds.[3–5] Its impact on stratospheric ozone is insignificant due to its rapid destruction by UV light. However, high manufacturing costs may limit its utility as a fumigant. Other soil fumigants currently in use, such as 1,3-dichloropropene (1,3-D), chloropicrin and metam-sodium, generally have narrower spectra of activity than methyl bromide, and thus a combination of these fumigants would have to be used to replace the latter. Increasing public concern about the possible adverse environmental impacts of these chemicals may incite more regulatory restrictions on their use. In California, the use of 1,3-D is currently limited to certain counties and by application rate restrictions.

Propargyl bromide (bromopropyne), originally developed by the Dow Chemical Company in the 1950s, was once tested as a fumigant. Its physical properties make it attractive as a soil fumigant.[6] Interactions of propargyl bromide with important soil-borne pathogens and pests are poorly understood. In this study, we evaluated the efficacy of propargyl bromide for the control of barnyardgrass (*Echinochloa crus-galli* (L) Beauv) and *Fusarium oxysporum* Schlecht in soils collected from regions where fumigation is routinely conducted. Such information is useful for establishing effective application rates for propargyl bromide.

* Correspondence to: Jianying Gan, Department of Environmental Sciences, University of California, Riverside, CA 92521, USA
E-mail: jgan@ucrac1.ucr.edu

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2 MATERIALS AND METHODS

2.1 Soils and fumigant

The Arlington sandy loam (coarse-loam, mixed, thermic, Haplic, Durixeralf) was sampled from the top 20 cm of a field at the University of California Agricultural Experiment Station in Riverside, CA. The soil consisted of 64% sand, 7% clay and 0.9% organic matter. The Carsitas loamy sand (sandy, mixed, typic, hyperthermic) was obtained from the top 20 cm of a field at the University of California Agricultural Experiment Station in Coachella, CA. The soil consisted of 84% sand, 5% clay and 0.8% of organic matter. Florida muck soil (Euic, hyperthermic Lithic Meisaprists) was collected from the Everglades Research and Education Center near Belleglade, FL. The soil consisted mostly of organic matter (78.2%). Fresh soils were passed through a 2-mm sieve and stored at room temperature until use. Technical standard propargyl bromide (97%) was purchased from Fluka (Buchs, Switzerland).

2.2 Fungi and weed seeds

Millet seeds were soaked in water in a flask for 2 h and then autoclaved twice, each time for 20 min with a 24-h interval. A pathogenic strain of *F oxysporum* was isolated from *Heterodera schachtii* Schmidt cysts in a diseased field at the University of California Agricultural Experiment Station in Riverside, CA. The fungi were grown on potato dextrose agar (PDA),[7] and then four to five pieces of the PDA were transferred into a 500-ml Erlenmeyer flask containing 500 g of millet seeds. The flask was shaken thoroughly and placed in the incubator for two weeks at 26 °C. During incubation, the flask was shaken every 2–3 days to obtain a uniform inoculation of the fungi on the seeds. The millet seeds were then dried in a laminar-flow hood under sterile conditions for 24 h and stored in a clean plastic bag at 5 °C before use. The barnyardgrass seeds were purchased from Valley Seed Service, Fresno, CA. The seeds were tested to give a germination rate of >99% at 22 °C.

2.3 Treatment and analyses

Fifty grams of the Arlington and Carsitas soils, and 20 g of the Florida muck, were weighed into 165-ml jars. The soils were adjusted to approximately 60% of field capacity, which was 6.8% (w/w) for the Arlington sandy loam, 6.2% for the Carsitas loamy sand and 70.7% for the Florida muck soil. Fungi on 30 millet seeds or 50 barnyardgrass seeds were mixed with the soil and pre-incubated for 24 h at room temperature (22 °C) before fumigation.

Stock solutions of various propargyl bromide concentrations were prepared by dissolving the chemical in ultra-pure water just before use. The soil samples were treated with propargyl bromide solutions to give different initial soil concentrations. On the basis of observations in preliminary experiments, much higher concentrations were adopted for the Florida muck soil than for the other two soils. Four replicates were used for each concentration. The treated soil jars were capped immediately with aluminum seals and Teflon-faced butyl rubber septa. The capped jars were then transferred to an incubator at 30 (±0.5) °C. This temperature was chosen to represent typical soil temperature in the upper crop root zone during fumigation in California and Florida. The experiments for fungus and weed seed treatments were carried out separately. Samples having had no propargyl bromide treatment served as control.

For the *F oxysporum* and barnyardgrass bioassays, the soil was sieved to recover the seeds. Ten *F oxysporum*-infested millet seeds were selected randomly from each sample and transferred onto a PDA growth medium in a sterile Petri dish at 22 °C. The Petri dishes were examined daily to monitor growth of the fungi. White restricted colonies of *F oxysporum* developed around the seeds, which later turned pink. Colonies were counted after 5 days. Thirty barnyardgrass seeds were randomly selected, transferred into a Petri dish containing a moist germination blotter, and incubated for 5 days at 22 °C, after which the germinated seeds were counted.

To evaluate the efficacy of propargyl bromide, a dose-response curve was constructed by plotting survival (%) as a function of the initial propargyl bromide concentration. The data were fitted to a logistic function to derive the effective concentration for 50% kill (LC_{50}). This index was used for comparing pest-control efficacy. The general form of the logistic function is:

$$r = \frac{a}{1 + \left(\frac{C_m}{C_{mr}}\right)^b} \qquad (1)$$

where r is percentage survival (100-mortality), C_m is total propargyl bromide concentration ($\mu g\ g^{-1}$) based on soil mass, and a and b are fitting parameters. For a perfect fit, when $a = 100$ (100% survival), C_{mr} is the soil concentration corresponding to 50% kill, or LC_{50}. Alternatively, LC_{50} can be calculated by setting $r = 50$ in eqn. (1). Similarly, the concentration for 90% kill (LC_{90}) can be calculated by setting $r = 10$ in eqn. (1).

Separate experiments were conducted to determine the degradation and adsorption of propargyl bromide in the soils, as these may significantly affect the efficacy of the compound. Ten grams of the Arlington or Carsitas soil, or 3 g of the Florida muck soil was weighed into a 21-ml glass headspace vial. Each vial was treated with propargyl bromide at an initial concentration of $6.8\ \mu g\ g^{-1}$. The treated vials were immediately sealed with aluminum caps and Teflon-faced butyl rubber septa, and transferred to a 30 °C incubator. At different time intervals three vials for each soil type were removed and stored at −20 °C until analysis. For analysis, sample vials were opened while the soil was still frozen, and 10 g of anhydrous sodium sulfate and 10 ml of ethyl acetate were quickly added. The vials were immediately recapped and shaken on a shaking machines for 1 h, followed by mixing on a vortex for 1 min. An aliquot of the supernatant from each vial was transferred into a gas chromatography (GC) vial. The concentration of propargyl bromide was quantified on a HP6890 GC equipped with a micro-electron capture detector. Preliminary studies showed that this extraction method gave an average recovery of 96% for propargyl bromide. The GC column was a 30 m × 0.25 mm × 1.4 μm RTX-624 capillary column (Restek Co, Bellefonte, PA), and the flow rate was 1.1 ml min^{-1} (helium). The oven temperature was initially 70 °C and then ramped up at 15 °C min^{-1} to reach 140 °C. The inlet temperature was 230 °C and the detector temperature 280 °C.

Propargyl bromide concentrations (C_m, μg g^{-1}) measured at different times (t; h) were fitted to first-order kinetics to obtain the first-order degradation rate constant (k).

$$C_\mathrm{m} = C_\mathrm{mo} e^{-kt} \tag{2}$$

where C_mo is the initial propargyl bromide concentration. The degradation half-life ($t_{1/2}$; h), which is the time at which $C_\mathrm{m} = 0.5 C_\mathrm{mo}$, was calculated from:

$$t_{1/2} = \frac{\ln 2}{k} \tag{3}$$

The $t_{1/2}$ value was used as a measure of propargyl bromide persistence.

A batch equilibration method was used to determine propargyl bromide adsorption to soil. Briefly, 5.0 g (dry weight) of the Arlington or Carsitas soil, or 3.0 g (dry weight) of the Florida muck soil was weighed into a 35-ml polypropylene centrifuge tube. Adsorption of propargyl bromide to the tube was determined to be small and was neglected. Propargyl bromide (2.72 mg litre^{-1}) in 0.1 M calcium chloride solution was added into the centrifuge tubes, with four replications for each soil type. Soil-less blank tubes were used as control. The capped tubes were shaken for 2 h on a reciprocal shaking machine at room temperature, and then centrifuged at 10 000 rev min^{-1} for 15 min at <10 °C. An aliquot (5.0 ml) of the supernatant was removed from each tube and extracted with ethyl acetate (10.0 ml). The extract was dried with anhydrous sodium sulfate (5.0 g), and a sample (1.5 ml) was transferred to a GC vial for analysis, using the GC method given above. The soil remaining in the centrifuge tube was further extracted with ethyl acetate, using the same steps as in the degradation study. The linear adsorption constant, K_d (cm^3 g^{-1}), was calculated from propargyl bromide concentrations in the solution phase (C_l, μg cm^{-3}) and on soil (C_s, μg g^{-1}):

$$K_\mathrm{d} = \frac{C_\mathrm{s}}{C_\mathrm{l}} \tag{4}$$

The activity of a fumigant to pests, pathogens or weeds depends on the concentration and time of exposure. The combined effect of both is often indicated by a concentration-time index (CT) which is calculated as the integral of concentration with respect to time:

$$CT = \int_0^t C_\mathrm{m} dt \tag{5a}$$

If C_m decreases exponentially with time and follows eqn (2), then eqn (5a) can be simplified as:

$$CT = \int_0^t C_\mathrm{m} dt = \frac{C_\mathrm{mo}}{k}[1 - \exp(-kt)] \tag{5b}$$

Although CT varies depending on the chemical and pest species, it has been reported that CT is relatively constant for a given pesticide and pest species within an effective concentration range of the pesticide in a given soil.[8–10] Therefore, CT can be used as an index to determine the minimal application rate for effective pest control.

3 RESULTS AND DISCUSSION

3.1 Dose-response relationships between propargyl bromide and barnyardgrass

Propargyl bromide concentration had a significant effect on germination of barnyardgrass seeds in the Arlington, Carsitas and Florida soils (Fig 1). Response of the grass seeds to propargyl bromide in all soils followed a general *s*-shape curve. Seed germination was not significantly inhibited at low concentrations, but the germination rate decreased with increasing

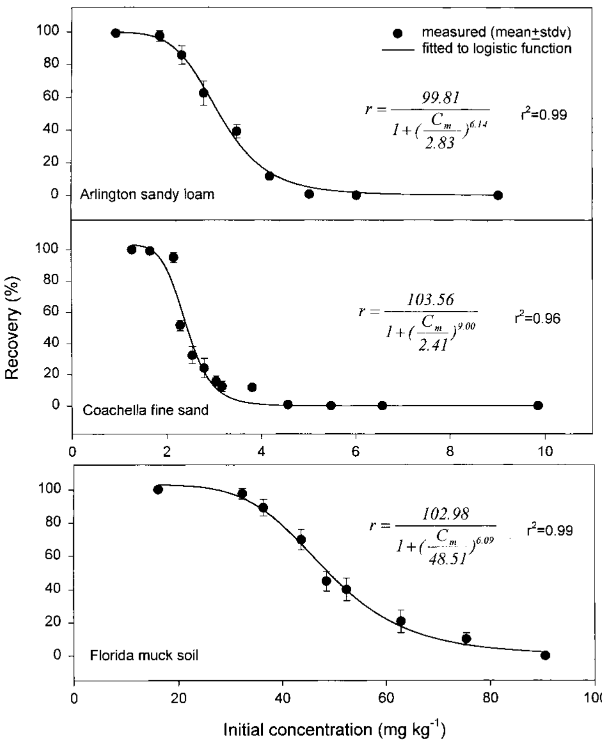

Figure 1. Dose-response relationships between propargyl bromide and barnyardgrass seeds in Arlington sandy loam, Carsitas loamy sand and Florida muck soil at 30 °C.

Table 1. Concentrations of propargyl bromide required to kill 50% (LC_{50}) and 90% (LC_{90}) of barnyardgrass seeds in Arlington sandy loam, Carsitas loamy sand and Florida muck soil, and propargyl bromide concentrations in soil solution at 0 ($C_l^{t=0}$) and 24 h ($C_l^{t=24}$) after treatment

Soil	θ ($cm^3 cm^{-3}$)	ρ ($\mu g cm^{-3}$)	K_d ($cm^3 g^{-1}$)	C_{mr} ($\approx LC_{50}$) ($\mu g g^{-1}$)	LC_{90} ($\mu g g^{-1}$)	$C_l^{t=0}$ ($\mu g\ cm^{-3}$)	$C_l^{t=24}$ ($\mu g\ cm^{-3}$)
Arlington	0.11	1.50	0.96	2.8	4.1	2.7	2.1
Carsitas	0.10	1.65	0.87	2.4	3.1	2.6	2.0
Florida	0.57	0.80	5.60	48.5	70.0	7.7	0.7

concentration, and no germination was observed at the high concentrations. This response pattern was described by the logistic function of eqn (1), with high regression coefficients for all soils ($r^2 > 0.95$) (Fig 1). Good fits allowed the use of the fitted equations for calculating LC_{50} values (Table 1). The LC_{50} for barnyardgrass was estimated to be 2.8, 2.4 and 48.5 $\mu g\ g^{-1}$ in the Arlington sandy loam, Carsitas loamy sand and Florida much soil, respectively (Table 1). Therefore, while propargyl bromide appeared to be equally effective for controlling barnyardgrass in the Arlington and Carsitas soils, higher concentrations were required to achieve the same control in the Florida muck soil. The fitted equations were also used to calculate LC_{90} values (Table 1). The estimated LC_{90} values (Table 1) further confirm that a much higher propargyl bromide application rate would be required for the Florida soil than for the Arlington and Carsitas soils. Based on the estimated LC_{90}, the application rates for propargyl bromide to achieve 90% weed control would be 12, 10 and 110 $kg\ ha^{-1}$ for the Arlington sandy loam, the Carsitas loamy sand and the Florida muck soil, respectively, assuming that the chemical was uniformly distributed in the top 20 cm of soil. This assumption tends to underestimate the chemical concentrations at the injection points and overestimate the concentrations in the fringe. However, since many more loss processes may occur in the field,[11] the calculated application rates are only suggestive for actual field application rates.

3.2 Dose-response relationships between propargyl bromide and *F oxysporum*

The response of *F oxysporum* to propargyl bromide in all soils followed a pattern similar to that observed for barnyardgrass (Fig 2), and the S-shaped response curve was also well described by eqn (1) ($r^2 > 0.99$). However, the LC_{50} for *F oxysporum* was approximately four times greater than that for barnyardgrass in the same soil (Table 2), suggesting that propargyl bromide would be more effective for controlling weeds than for controlling *F oxysporum*. In studying dose-response relationships between methyl bromide and a range of fungi, Minuto *et al*[12] observed that *F oxysporum* was the most difficult pathogen to control. In this study, the LC_{50} values for *F oxysporum* were similar in the Arlington sandy loam and the Carsitas loamy sand, and about 17 times smaller than that in the Florida muck soil. The calculated LC_{90} values show a similar trend (Table 2). The LC_{90} values would correspond to application rates of 37, 43 and 360 $kg\ ha^{-1}$ in the Arlington, Carsitas and Florida soils, respectively, subject to the same assumptions as used for barnyardgrass.

3.3 Effects of propargyl bromide degradation and adsorption on its efficacy

The low activity of propargyl bromide in the Florida muck soil could result from a number of factors. The dose-response curves for barnyardgrass (Fig 1) and *F oxysporum* (Fig 2) in this soil suggest that the effective propargyl bromide concentrations available to interact with the weed seeds and fungi may be low. This could be caused by rapid degradation and/or strong adsorption of the compound in the organic matter-rich soil. The degradation experiments showed that propargyl bromide concentration decreased exponentially with time in each soil and the degradation data were well described by first-order kinetics as shown in eqn (2) ($r^2 > 0.98$) (Fig 3). The first-order degradation rate constants (k) were 0.012, 0.010 and 0.10 h^{-1} in the Arlington, Carsitas and Florida soils, respectively. These k values correspond to $t_{1/2}$ values of 58, 69 and 7 h in the respective soils. Therefore, propargyl

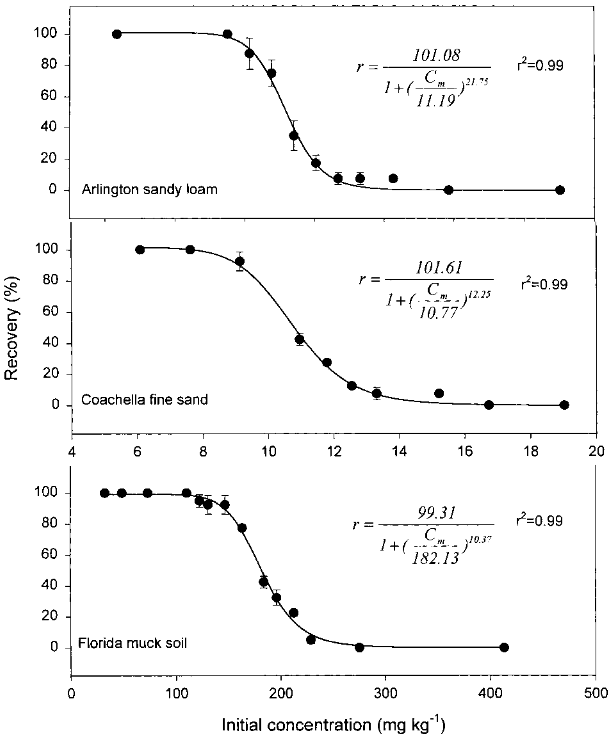

Figure 2. Dose-response relationships between propargyl bromide and *Fusarium oxysporum* in Arlington sandy loam, Carsitas loamy sand and Florida muck soil at 30 °C.

Table 2. Concentrations of propargyl bromide required to kill 50% (LC_{50}) and 90% (LC_{90}) of the inoculated *Fusarium oxysporum* in Arlington sandy loam, Carsitas loamy sand and Florida muck soil, and propargyl bromide concentrations in soil solution at 0 ($C_l^{t=0}$) and 24 h ($C_l^{t=24}$) after treatment

Soil	θ ($cm^3 cm^{-3}$)	ρ ($\mu g cm^{-3}$)	K_d ($cm^3 g^{-1}$)	C_{mr} ($\approx LC_{50}$) ($\mu g g^{-1}$)	LC_{90} ($\mu g g^{-1}$)	$C_l^{t=0}$ (μg cm^{-3})	$C_l^{t=24}$ (μg cm^{-3})
Arlington	0.11	1.50	0.96	11.2	12.4	10.8	8.2
Carsitas	0.10	1.65	0.87	10.8	12.9	11.6	9.1
Florida	0.57	0.80	5.60	182.1	225.0	28.9	2.6

bromide degraded about 10 times faster in the Florida soil than in the other two soils. On the basis of the CT principle, the higher rate of propargyl bromide degradation in the Florida soil suggests that a much higher initial concentration would be required to achieve similar control in the Florida soil than in the other soils. A correlation analysis showed that there was a high, negative correlation between $t_{1/2}$ and LC_{50} for both barnyardgrass and *F oxysporum* ($r^2 > -0.99$).

High soil organic matter content may also result in strong adsorption of organic compounds.[13,14] The equilibrium adsorption coefficients (K_d) of propargyl bromide were 0.96, 0.87 and 5.6 $cm^3 g^{-1}$ in the Arlington, Carsitas and Florida soils, respectively. It is commonly believed that adsorbed pesticides have little or no biological activity.[15] Therefore, the strong adsorption of propargyl bromide may have also contributed to its poor efficacy in the Florida soil. Correlation analysis showed that K_d and LC_{50} were highly correlated ($r^2 > 0.99$).

Assuming that only propargyl bromide in the solution phase was responsible for inhibiting barnyardgrass and *F oxysporum*, the observed efficacy of propargyl bromide in the soils (Figs 1 and 2) can be explained from the respective C_l values (Tables 1 and 2). Values of C_l in the tables were calculated from the following relationship:

$$C_l = \frac{C_v}{\theta + \rho K_d} \qquad (6)$$

where C_v ($\mu g cm^{-3}$) is total propargyl bromide concentration based on soil volume and calculated by multiplying soil bulk density by C_m, θ is the volumetric soil water content ($cm^3 cm^{-3}$), ρ is soil bulk density ($g cm^{-3}$), and C_l and K_d are as defined previously. In calculating C_l, the fraction of propargyl bromide in the gas phase was ignored because the reported air–water partitioning coefficient[6] was very small compared to K_d. Two sets of C_l values were calculated for the propargyl bromide concentrations corresponding to LC_{50} (Tables 1 and 2). The $C_l^{t=0}$ corresponds to the propargyl bromide concentration in soil solution at the beginning of the incubation, while $C_l^{t=24}$ is the concentration in soil solution after 24 h of incubation. Although the treated concentration in the Florida muck soil was 20 times greater than in the other two soils, the initial concentration in the solution phase was only three times greater (Tables 1 and 2) due to the stronger adsorption of propargyl bromide in the muck soil. The C_l in the Florida soil also decreased much more rapidly than in the other soils, and at 24 h after treatment, the C_l in the Florida soil was only a third of those in the other two soils. Therefore, the poor efficacy of propargyl bromide in the Florida soil was clearly attributable to both strong adsorption and rapid degradation of the compound in this soil.

Data in Fig 4 show the *CT* for propargyl bromide to achieve 50% barnyardgrass control in 24 h, or CT_{50}. The curves were generated using the propargyl bromide degradation rate constant (k) from different soils at an initial concentration corresponding to the LC_{50}, assuming that k does not change with the initial concentration. The *CT* values for the Arlington and

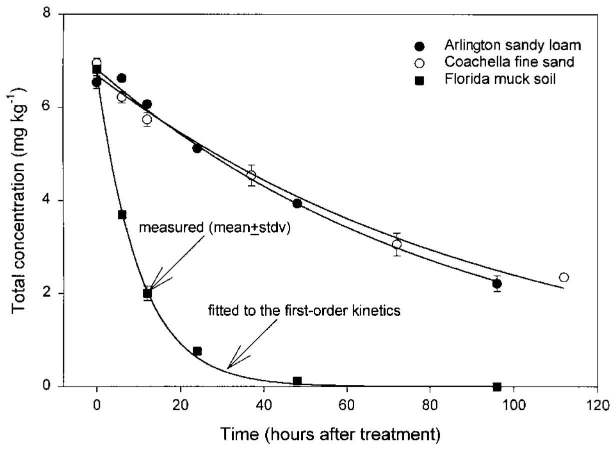

Figure 3. Degradation of propargyl bromide in Arlington sandy loam, Carsitas loamy sand and Florida muck soil at 30 °C at an initial concentration of 6.8 $\mu g g^{-1}$.

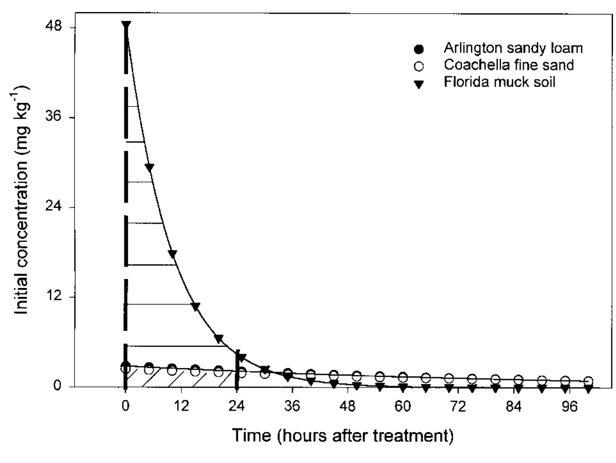

Figure 4. Concentration-time index required for propargyl bromide to achieve 50% control of barnyardgrass in 24 h in Arlington sandy loam, Carsitas loamy sand and Florida muck soil at 30 °C.

Carsitas soils were similar, and considerably smaller than that for the Florida soil (Fig 4). This suggests that higher propargyl bromide concentrations would be required to achieve the same efficacy against barnyardgrass in the Florida soil than in the other soils. Calculation using eqn (5b) gave CT_{50} values of 59, 51 and 442 $\mu g\,g^{-1}\,h$ for barnyardgrass in the Arlington, Carsitas and Florida soils, respectively. Likewise, CT_{50} values for *F oxysporum* were 235, 229 and 1660 $\mu g\,g^{-1}\,h$ for the corresponding soils.

4 CONCLUSIONS

The efficacy of propargyl bromide against barnyardgrass and *F oxysporum* varied significantly among different soils. While the compound showed similar efficacy in the sandy-textured Arlington sandy loam and Carsitas loamy sand, it was 20 times less effective in the organic matter-rich Florida muck soil. The low efficacy of propargyl bromide in the latter soil was attributable to the rapid degradation and strong adsorption of the chemical in the soil. In the same soil, the LC_{50} for barnyardgrass was approximately four times lower than the LC_{50} for *F oxysporum*. The estimated propargyl bromide application rates, based on the LC_{90}, were 10 and 50 $kg\,ha^{-1}$ for barnyardgrass and *F oxysporum* in the Arlington and Carsitas soils, and 112 and 360 $kg\,ha^{-1}$ in the Florida muck soil. This rate for the sandy soils is only a fraction of the average methyl bromide application rate (240 $kg\,ha^{-1}$). These estimates, however, were obtained under closed conditions, and the actual field rate may be substantially greater because of additional pathways for fumigant dissipation. Field experiments are needed to verify these results.

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